The Architecture and Chemical Stability of the Archaeal Sulfolobus Turreted Icosahedral Virus

Reza Khayat,1 Chi-yu Fu,1 Alice C. Ortmann,2,3 Mark J. Young,4,5,6,7*, and John E. Johnson1*

The Scripps Research Institute, Department of Molecular Biology, La Jolla, California1; Marine Sciences, University of South Alabama, Mobile, Alabama2; Dauphin Island Sea Lab, Dauphin Island, Alabama3; and Thermal Biology Institute Department of Plant Sciences and Plant,4 Center for Bioinspired Nanomaterials,5 Department of Plant Sciences and Plant Pathology,6 and Department of Chemistry and Biochemistry and Department of Microbiology,7 Montana State University, Bozeman, Montana

Received 2 April 2010/Accepted 18 June 2010

Viruses utilize a diverse array of mechanisms to deliver their genomes into hosts. While great strides have been made in understanding the genome delivery of eukaryotic and prokaryotic viruses, little is known about archaeal virus genome delivery and the associated particle changes. The Sulfolobus turreted icosahedral virus (STIV) is a double-stranded DNA (dsDNA) archaeal virus that contains a host-derived membrane sandwiched between the genome and the proteinaceous capsid shell. Using cryo-electron microscopy (cryo-EM) and different biochemical treatments, we identified three viral morphologies that may correspond to biochemical disassembly states of STIV. One of these morphologies was subtly different from the previously published 27-Å-resolution electron density that was interpreted with the crystal structure of the major capsid protein (MCP). However, these particles could be analyzed at 12.5-Å resolution by cryo-EM. Comparing these two structures, we identified the location of multiple proteins forming the large turret-like appendages at the icosahedral vertices, observed heterogeneous glycosylation of the capsid shell, and identified mobile MCP C-terminal arms responsible for tethering and releasing the underlying viral membrane to and from the capsid shell. Collectively, our studies allow us to propose a fusogenic mechanism of genome delivery by STIV, in which the dismantled capsid shell allows for the fusion of the viral and host membranes and the internalization of the viral genome.

Viruses are valuable biological tools for manipulating the cellular processes of their hosts, and they can also serve as model systems for describing macromolecular interactions through the analysis of their architecture. The Sulfolobus turreted icosahedral virus (STIV) is an archaeal virus that infects Sulfolobus solfataricus (phylum Crenarchaeota). STIV is a lytic virus that was isolated from an acidic hot spring (>80°C and pH of <3) in Yellowstone National Park (27). Hence, STIV is an important model for studying the biochemical requirements to sustain life in extreme physicochemical conditions and has the potential to become a tool for the biochemical and genetic manipulation of its host—much like bacteriophages lambda, P22, and phi29 have done for their respective hosts.

Prior structural studies of STIV using cryo-electron microscopy (cryo-EM), X-ray crystallography, and proteomics have described large pentameric turret-like structures, with petal-like protrusions emanating from their central shafts (27). The T=31d capsid shell is composed of trimeric capsomers exhibiting pseudo-hexagonal symmetry, in which each of the three capsomer subunits donates two viral jelly rolls with its β-sheets normal to the capsid surface (15, 27). Capsomers surrounding the icosahedral 3-fold axes, and their neighboring subunits, make direct contact with the viral membrane via a highly basic C-terminal helix of each subunit (15, 22). Surrounding the base of the turrets are proteins that make contact with the capsid shell and a host-derived viral membrane (15). The viral membrane and the enclosed viral genome are referred to as the lipid core.

The capsid architecture of STIV and the crystal structure of its major capsid protein (MCP) are strikingly similar to those of the bacteriophages PRD1, Bam35, and FM2, the alga virus PBCV-1, and the mammalian adenovirus. This similarity suggests that these viruses share an ancestral virus (2, 4, 7, 15, 25). Given the evolutionary relationship shared between STIV and PRD1, we postulated that the large turret-like vertices of STIV were used to inject the viral genome into the Sulfolobus host—a genome delivery mechanism employed by PRD1 (27).

A recent report by Brumfield et al. (5) describes gross cellular ultrastructural changes induced in the Sulfolobus host during STIV infection and release. The authors identified distinct particles that appear to be assembly intermediates of STIV en route to maturation. From these intermediates the authors proposed a general mechanism of capsid assembly, in which MCP subunits and minor capsid proteins (mCPs) coassemble with the lipid membrane to form a lipid-enclosed protein vesicle. These vesicles are spherical and lack the double-stranded DNA (dsDNA) genome and turret-like appendages at the vertices.

While these studies confirm an empty procapsid intermediate, the corresponding molecular mechanism associated with
assembly and disassembly remains to be understood. Moreover, little is known about STIV or other archaeal virus genome delivery into the host. To obtain a better understanding of the molecular mechanism of STIV architecture and its role in genome delivery, we characterized three distinct morphologies of STIV particles using cryo-EM. An image reconstruction of one of these revealed the absence of a number of constituents decorating the STIV capsid. Hence, for simplicity, we refer to the previously reported image reconstruction (27) as “decorated” and the new image reconstruction reported here as “undecorated.” Reference-free two-dimensional (2D) class averages of the second identified morphology reveal a partially decorated STIV lipid core. The third identified morphology corresponds to the isolated STIV lipid core. Taken together, our analyses indicate that these morphologies correspond to different disassembly intermediates of STIV that can be isolated in vitro and help provide a picture of the STIV capsid architecture. Additionally, these morphologies allow us to propose an alternative possible mechanism of genome delivery.

MATERIALS AND METHODS

Virus production. *S. solfataricus* strain 2.2-12 cultures were infected with STIV as previously described (Maaty et al. [23]). *Sulfobacillus* cells were grown from glycerol stock in medium 182 at pH 3.5. The cultures were passed twice to medium 182 at pH 3.5 and were then passed to 1L medium at pH 2.5. At an optical density at 650 nm (OD$_{650}$) of ~0.4, the cells were infected with an STIV multiplicity of infection (MOI) of ~2 and incubated for 72 h. Virus particles were collected from the culture medium after removal of cellular debris via centrifugation at 6,000 rpm for 10 min. The supernatant was filtered through a vacuum-driven filtration system (Steritop; Millipore) and concentrated to about 2 ml with centrifugation filter units with a 100-kDa cutoff (Amicon Ultra-100; Millipore). The concentrated supernatant was applied to the 38% (wt/vol) CsCl$_2$ for the preparation of “disassembled” particles (see Results and Discussion). The samples were centrifuged at 40,000 rpm overnight using a Beckman Coulter SW41 Ti rotor. The viral particles were recovered from the gradients and dialyzed against citric buffer. The purified particles were concentrated to ~10 mg/ml.

Data collection and cryo-EM image reconstruction. Frozen hydrated samples of STIV were prepared on C-flat CF-22-4C grids (Protochips). Briefly, a 4-µl sample of the purified virus was applied to the grid, blotted for a short period of time, and dipped into a liquid ethane bath using an FEI Vitrobot instrument. Samples were stored and handled in liquid nitrogen. Data were collected at the National Resource for Automated Microscopy using the LEGINON suite operating an FEI Tecnai F20 electron microscope at 120 kV. Vitrified samples were observed under liquid nitrogen temperatures using low-dose conditions. Images were captured on a Gatan 4K-by-4K-pixel charge-coupled device (CCD). Images used for the image reconstruction were collected, and the nominal magnification of ×80,000, with defocus values from −1.5 to −2.5 µm (30). The pixel size of the CCD was calibrated using the defraction pattern from a 2D catalase crystal. Contrast transfer function (CTF) estimation and correction were done using automated CTF estimation (ACE) through the Appion package (19, 24). A spherical shell with the size and thickness of the type I particles (measured from the micrographs) was used as the starting model for the undecorated image reconstruction. The image reconstruction was generated with EMAN (22). The resolution of the image reconstruction, as determined by Fourier shell correlation (FSC) of 0.5, was 12.5 Å.

Reference-free classification. The CTF corrected images were low-pass filtered to 15 Å with EMAN prior to the analysis. Reference-free alignment of type II particles was obtained using rotational power spectra with the program XMIPP (29). The rotational power spectra were classified using 7-by-7 self-organizing maps (KerDenSOM), with an initial smoothness factor of 500, a final smoothness factor of 100, and 5 deterministic annealing steps using XMIPP.

Reciprocal space refinement of the pseudo-atomic model. The orientation of the undecorated image reconstruction was changed from the EMAN to the VIPER convention, and the handedness was adjusted to the dextro configuration. The MCP subunits (lacking the 26 C-terminal residues) were docked into the image reconstruction as previously described (15). The lipid core was masked from the image reconstruction using a spherical mask. Structure factors for the capsid shell were calculated to a 12.5-Å resolution using the crystallography and nuclear magnetic resonance (NMR) system (CNS) (6). Rigid-body refinement (in which each subunit is treated as a rigid body) with CNS improved the fit of the MCP model. A temperature factor (B-factor) sharpening value for the image reconstruction was determined using an iterative process of density sharpening followed by reciprocal space refinement. Briefly, the unmasked image reconstruction was sharpened by −50 Å$^2$ increments from 0 to −1,000 Å$^2$ and processed using the method described above. A sharpening factor of −300 Å$^2$ produced the lowest R and $R_{free}$ values, against an MCP pseudo-atomic shell model possessing a global B-factor of 500 Å$^2$. A mask of the MCP shell was calculated using a 15-Å probe around all atoms of the refined model and applied to the sharpened map to produce the density for the MCP shell. Structure factors were calculated from the MCP shell density and used for subsequent rounds of rigid-body and B-factor refinement. An overall B-factor of 500 Å$^2$ for the model produced the lowest R and $R_{free}$ values. An additional round of grouped B-factor refinement (2 groups per residue, main chain and side chain) was carried out with the minimum and maximum B factors set to 300 and 700 Å$^2$, respectively. The B-factor of the resulting model was smoothed several times with MOLEMAN (17).

Difference map calculation. A difference map between the decorated and the undecorated reconstructions was obtained by low-pass filtering the undecorated to the resolution of the decorated reconstruction (27 Å). The scale of the decorated reconstruction was adjusted to produce a radial plot similar to the radial plot of the undecorated reconstruction. The pixel size between the two reconstructions was made equivalent by trilinear interpolation of the decorated reconstruction with SPIDER (8). The two image reconstructions were then cropped to the same cell dimensions with the EMAN package. The aligned reconstructions were scaled to have similar average, standard deviation, and minimum and maximum density values and subtracted from one another using MAPMAN (17).

The difference map between the B-sharpened undecorated reconstruction and refined MCP pseudo-atomic density was calculated by subtracting the calculated MCP electron density from the undecorated electron density using the method described above.

RESULTS

Data collection and particle description. Several cryo-EM datasets of STIV particles purified from different preparations have been collected, and three distinct particles in the micrographs can be observed. Type I particles areicosahedral, type II particles are nodular, and type III particles are spherical (Fig. 1). The diameters of the type I, II, and III particles (measured directly from the micrographs) are ~970 Å, ~860 Å, and ~510 Å, respectively. All three particles possess a similarly sized spherical ultrastructure that contains two electron-dense layers at the perimeter (Fig. 1). The spherical ultrastructure is the lipid core of type I particles. Type II particles exhibit components decorating the spherical ultrastructures but lack the capsid shell and large turrets clearly visible in the type I particles. Type III particles are the spherical ultrastructure. Particles pertaining to each type are manually extracted from the CTF-corrected micrographs and subjected to image analysis (Table 1).

Type I particle cryo-EM image reconstruction. The cryo-EM image reconstruction of type I particles shows a number of attributes similar to those of the earlier reported cryo-EM image reconstruction (27). Briefly, the turret-like vertices project ~110 Å from the ~100-Å-thick capsid shell. Within each turret, along the 5-fold axis, is an ~40-Å-wide channel that is capped at opposite ends by density. Densities capping the two ends of the channel disappear at contour levels greater than 70% MCP mass content (2r), such that the channel traverses from inside the virus particle to the outside. The T = 31d capsid
shell encloses a host-derived single-layered membrane (23).

Concurrently there are a number of prominent features that distinguish the type I image reconstruction from the previously published image reconstruction. These differences help define the boundaries for constituents that define the architecture of STIV. The petal-like densities that could be seen decorating the tips of the turret-like vertices in the previous image reconstruction are not observed with the type I particle image reconstruction (Fig. 2A). A difference map calculated between the two image reconstructions helps define the molecular boundaries for the constituents of this density (Fig. 2B and C). Each petal-like density corresponds to an approximate mass of 63 kDa. It was previously postulated that the constituent of this density was the gene product of open reading frame (ORF) C557 (59.4 kDa) (23). The image reconstruction of type I particles strongly supports this assignment, and the lack of an observable protein band for C557 in the Coomassie blue-stained SDS-PAGE gel of purified type I particles further supports this assignment (Fig. 1). Interestingly, traces of the petal-like densities can be visualized in the type I particle image reconstruction at contour levels lower than 0.3 σ, indicating partial occupancy of the turrets by the constituents of these densities. For simplicity, we refer to the previously published and type I particle image reconstructions as decorated and undecorated, respectively.

An additional difference between the two reconstructions are the feet-like densities (19 kDa) that can be seen surrounding the base of the vertices in the difference map calculated by subtracting the MCP pseudo-atomic shell model from the decorated image reconstruction (Fig. 2B). The densities contact the turrets, the MCP shell, and the viral membrane—interactions reminiscent of the transmembrane protein P16 in PRD1 and unidentified proteins surrounding the base of the adenovirus and PM2 vertices (2, 15). These densities are not observed with the undecorated image reconstruction, and the turrets do not contact the viral membrane. Similarly, the finger-like densities that could be seen connecting the MCP shell to the viral membrane of the decorated image reconstruction are lacking in the undecorated image reconstruction. Instead, the viral membrane contracts by ~10 Å into the interior of the undecorated image reconstruction capsid (Fig. 3A and 4B). Contraction of the viral membrane may be explained by its physicochemical properties. The STIV viral membrane is a fully saturated long hydrocarbon and therefore, at reduced temperatures, may solidify and contract—much like the solidification and contraction of melted butter at room temperature.

Low-pass filtering the undecorated image reconstruction to 27 Å or generating an image reconstruction using the same number of undecorated particles and procedure used to generate the decorated image reconstruction produces results similar to that described above, demonstrating that the morphological difference between the decorated and undecorated particles is not due to the image reconstruction process.

The turret-like appendages of STIV. Proteins belonging to ORFs C381 and A223 are absent in the type II (disassembled) particles. Proteins belonging to ORFs C381 and A223 are absent in the type II (disassembled) particles.
base of each turret corresponds to five copies of the ORF A223 gene product (Fig. 2; see also Discussion).

**Mobile arms and the viral membrane.** Two concentric layers of density peaks exterior to the viral membrane are evident in the difference map calculated by subtracting the density of the pseudo-atomic MCP shell from that of the undecorated image reconstruction. Directly exterior to the viral membrane and radiating outward are finger-like densities that reach into the

---

**FIG. 2.** Surface representations of the STIV cryo-EM image reconstructions contoured at 100% MCP mass content (1.1 $\sigma$). (A) Decorated and undecorated image reconstructions on the left and right, respectively. The turret-like vertices are colored yellow, the MCP shell cyan, the lipid layer red, and the genome blue. The genome in the undecorated image reconstruction cannot be visualized at the rendered contour threshold. (B) The segmented turret-like vertices of the decorated and undecorated image reconstruction on the left and right, respectively. Colored in yellow, magenta, and blue are the densities assigned to proteins from ORFs A223 and C381 (indicated on the figure), C557, and A55/B130, respectively. (C) Segmented densities for the petal- (magenta) and feet-like (blue) regions of the vertices. These densities pertain to substituents with masses of 63 and 19 kDa and are assigned to the proteins from ORFs C557 and A55/B130, respectively.

---

**FIG. 3.** Surface representation of difference maps calculated from the undecorated image reconstruction. Densities for the genome, viral membrane, and vertex turrets are contoured at 1.1 $\sigma$, and densities for the MCP region are contoured at 1.7 $\sigma$. The higher contour threshold for the MCP region is to visually reduce the noise present in the difference maps. (A) Outline of an icosahedral facet. Colored in red is the viral membrane, in blue are the finger-like densities, in yellow are the vertex turrets, and in gold and outlined in black are the glycosylation sites. Two of the iASUs are outlined with red and cyan polygons. The letters identify the five capsomers in the iASU. (B) Difference map comparison of the decorated and undecorated image reconstructions. (C) Side view of capsomer E overlaid with the undecorated difference map. The MCP C-terminal helix has been modeled into finger-like densities (blue).


cavern formed within the base of each capsomer. Further out from these peaks are sets of globular peaks (Fig. 3A and B).

In the decorated image reconstruction we modeled the highly basic 26 C-terminal residues of the MCP (2.7 kDa) as a helix that interacted with the viral membrane. In the undecorated image reconstruction difference map, finger-like densities (one per MCP subunit) are situated close to the unmod-

erated undecorated and type II particles produce radially averaged density plots with similar features in the lipid core structure for the density peaks near the icosahedral 3-fold cor-

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.2</td>
<td>1.2</td>
<td>1.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.6</td>
<td>1.1</td>
<td>1.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.3</td>
<td>2.2</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.2</td>
<td>1.2</td>
<td>1.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A single subunit from each refined pseudo-atomic model capsomer was aligned onto the indicated refined capsomer. The root mean square deviation (RMSD) was calculated for the remaining four nonaligned subunit Ca atoms. Both alignment and RMSD calculations were done with the UCSF Chimera package. Values are expressed as Å.

The STIV capsid shell is composed of a number of distinct gene products, with the MCP present in the greatest number of copies. The MCP is monomeric in solution and remains so even in the confines of a crystal lattice, suggesting that the trimeric capsomers, as seen in the virion capsid, may be an unstable oligomer (15). The heterogeneous geometries of the five capsomers in the iASU appear to support this (Table 2). This is in contrast to the evolutionarily related ade-novirus, PBCV-1, PRD1, PM2, and Bam35, in which the trimeric capsomers are more stable than their capsids and thus likely to form prior to being incorporated into the capsid. The interaction between the STIV MCP and viral membrane may be responsible for forming and stabilizing the STIV capsomers.

The pseudo-atomic models and difference maps of the deco-
rated and undecorated cryo-EM image reconstructions show that contacts between neighboring capsomers are limited to the two viral jelly roll domains of each MCP subunit. The N terminus of the MCP (residue Gly2) is buried within the trimer interface of each capsomer and cannot contribute to the capsomer-capsomer interaction. In the decorated image recon-


duction, density for the C terminus of capsomers near the center of the icosahedral facet can be seen connecting the MCP shell to the viral membrane; however, density for the C terminus could not be identified for the subunits at the edges of the facet (15). The MCP C termini in the undecorated image reconstruction nestle between the viral jelly rolls of their respective MCP subunits and do not contribute to capsomer-
capsomer interactions. Therefore, the orientations of the C termini in the two reconstructions suggest that the MCP C terminus may not be involved in contacting neighboring cap-
somers. This is in sharp contrast to the intimate involvement of the N and C termini of the major capsid proteins of PRD1, Bam35, and PM2 in forming capsomer-capsomer contacts. In fact it is this interaction, controlled via extensive N- and C-terminal conformational switching throughout the iASU, that is believed to be the driving force for the proper capsid assembly of PRD1, Bam35, and PM2 (1, 2, 12, 20, 28).

Some viruses use minor capsid proteins (mCPs) as cementing proteins to stabilize the capsid shell. Proteins such as the tape measure protein P30 of PRD1 and its homologues in Bam35 and adenovirus, the P3 and P6 proteins of PM2, and protein VIII in adenovirus serve such a function. Proteomic analysis of purified STIV has identified two mCPs (gene products of ORFs A55 and B130) with long predicted transmembrane helices that are likely to interact with the STIV viral membrane (23). These proteins may be the constituents of the feet-like densities seen at the base of the turret-like vertices in the decorated image reconstruction (Fig. 2B and C). These densities make contact with the viral membrane, the turret-like vertices, and the MCP shell. The interaction between the viral membrane, the mCPs, the turret-like vertices, and the C termini of the MCP subunits may be responsible for partially stabilizing the STIV capsid shell.

Thin sections of STIV-infected S. solfataricus cells reveal that capsid formation involves capsid proteins assembling around the viral membrane (5). SDS-PAGE analysis of undecorated and disassembled STIV particles shows that minor capsid proteins remain attached to the lipid core of both of these particles (Fig. 1). Therefore, it is highly plausible that the viral membrane and its associated minor capsid proteins serve as a scaffold for both the assembly and stability of the STIV capsid shell. In this regard, the MCP subunits form trimeric capsomers as a result of their interaction with the viral membrane and or minor capsid proteins. This would explain why recombinantly expressed MCP capsomers remain monomeric even in the confines of a crystal lattice. Glycosylation of the MCP subunits could further help stabilize capsomers prior to or after their incorporation onto the viral membrane.

We believe that the lack of observable interaction between the viral membrane and the capsid shell in the undecorated image reconstruction may result from the icosahedral symmetry imposed during the image reconstruction. Interactions between the capsid shell and the viral membrane, whether it be a direct interaction or an interaction mediated through mCPs, that do not follow icosahedral symmetry would be averaged out of the image reconstruction and therefore unobservable.

A recent report by Happonen et al. (11) describes the image reconstruction and architecture of an S. solfataricus virus that is homologous to STIV, annotated as STIV2. Intriguingly, the image reconstruction of STIV2 is nearly indistinguishable from the undecorated image reconstruction of STIV, in which the petal-like densities are missing from the vertex complex. Happonen et al. report that sequence comparison of the STIV and STIV2 genomes identifies ORF B631 of STIV2 as the homologue of the STIV ORF A223, with a 408-residue insertion, and ORF C510 of STIV2 as the homologue of the STIV ORF C557. The authors argue that there is no homologue of the
STIV ORF C381 in the genome of STIV2. Given the lack of petal-like densities decorating the turrets of the STIV2 image reconstruction and absence of the STIV ORF C381 from the STIV2 genome, Happonen et al. go on to argue that the turrets of STIV2 are composed of the gene products of B631 and C510 and that ORF C381 of STIV is the constituent of the STIV petal-like densities. This is clearly in sharp contrast to our assignment of proteins A223 and C381 composing the STIV turrets and protein C557 composing the petal-like densities. Given the sequence homology shared between the STIV and STIV2 structural proteins, the turrets of these two viruses likely share a conserved architecture.

A closer inspection of the STIV2 ORF B631 sequence reveals that this gene is, in fact, a fusion of the STIV A223 and C381 sequences, rather than the deletion of the STIV C381 gene described by Happonen et al. The ORF C381 is directly at the 3' end of ORF A223 in the genome. Sequence alignments of the gene products from the STIV2 ORF B631 and a fused version of the STIV ORF A223/C381 using the PROMALS server reveals that the two proteins share 36% sequence identity and 52% sequence similarity using the Blosum62 matrix (Fig. 5) (26). The gene products of the STIV ORFs A223 and C381 align to the N and C termini of the STIV2 ORF B631, respectively, with 41 and 33% sequence identity; therefore, STIV2 has a protein equivalent to the STIV ORF C381. This suggests that the assignment of the STIV ORF C381 to the petal-like densities of the STIV turrets by Happonen et al. is inaccurate. The petal-like densities of STIV ORF C381 to the petal-like densities of the STIV turrets by Happonen et al. is corroborated by the presence of the STIV MCP towers (Fig. 2C) (23). Additionally, glycosylation of MCP subunits may help stabilize capsomer formation. The tapering of density strength in the difference peaks, with respect to the icosahedral 3-fold axis and the edge of the icosahedral facets, is corroborated by proteomic data that shows the MCP subunits to be heterogeneously glycosylated (23). The difference map calculated from the undecorated image reconstruction and the MCP pseudo-atomic model show difference peaks near the MCP towers (Fig. 3C). Glycosylation of the STIV MCP towers is likely to help cope with the extreme physico-chemical extracellular environment. Additionally, glycosylation of MCP subunits may help stabilize capsomer formation.

The glycosylation of proteins has been documented as a strategy to guide proper protein folding, protect against proteolysis, and increase stability (13). Proteomic analysis of purified STIV revealed the MCP subunits to be heterogeneously glycosylated (23). The difference map calculated from the undecorated image reconstruction and the MCP pseudo-atomic model show difference peaks near the MCP towers (Fig. 3C). Glycosylation of the STIV MCP towers is likely to help cope with the extreme physico-chemical extracellular environment. Additionally, glycosylation of MCP subunits may help stabilize capsomer formation. The tapering of density strength in the difference peaks, with respect to the icosahedral 3-fold axis and the edge of the icosahedral facets, is corroborated by proteomic data that shows the MCP subunits to be heterogeneously glycosylated (23). Heterogeneous glycosylation of proteins regularly occurs in nature, particularly in the case of viral envelope glycoproteins, where it serves to evade an immune response (18).

Three different types of particles can be seen in the micrographs collected for STIV purified under different conditions (Fig. 1). Image analysis of these particles revealed STIV in differing states of disassembly. STIV grows at high temperatures (>80°C) yet, due to technical limitations, is handled at room temperature for purification and characterization. Although both decorated and undecorated samples were subject to this treatment, the turrets and membrane are different in the two samples. Since at a low contour level there is some decoration (low occupancy of the petals) of the undecorated particles, the difference in the two particle types appears to be continuous and not binary. We propose that the decorated particles correspond more closely to the high-temperature form of the virus, while the undecorated particles reflect changes that occur at lower temperature. We are at a loss to explain our inability to capture the highly decorated form of the particles in our recent preparations for cryo-EM analysis.

Purification of STIV using a CsCl2, rather than a CsSO4, gradient produces the disassembled particles, indicating that CsCl2 is a chaotrope for STIV (Fig. 1).
Although it is premature to discern whether these particles are physiologically relevant or simply a biochemical product of the purification procedure of STIV, the particles do shed light on the biochemical properties of STIV. The absence of a capsid shell in type II and III particles strongly suggests that the lipid core does not rely on the capsid shell for stability and that it may be isolated in vitro. A self-sufficient lipid core may indicate how STIV delivers its genome into the Sulfolobus host.

Bacteriophages PRD1 and PM2 and the animal adenovirus are evolutionarily related to STIV. These viruses are better characterized and may therefore act as guides for understanding STIV. PRD1 and PM2 contain a genome-enclosed lipid core that is crucial for infection, yet each bacteriophage utilizes this membrane via a distinct mechanism to deliver its genome. PRD1 delivers its genome by injecting it through a lipid membrane conduit that forms at a vertex from the fusion of the PRD1 lipid membrane to the host lipid membrane (9). In contrast, PM2 dismantles its capsid shell to allow the exposed lipid core to fuse with the host’s lipid layer and deliver the genome into the host (16). Similarly, adenovirus undergoes a dismantling process of the capsid shell to allow for the transfer of the genome across the nuclear pore complex (10).

We had originally proposed that STIV injects its genome into its host through one of its turret-like vertices. This hypothesis was based on the structural analogy of the STIV turret-like vertices to the vertex tails of bacteriophages responsible for injecting the phage genome into the host, and the evolutionary relationship between STIV and bacteriophage PRD1 (27). However, the recent structural characterization of the bacteriophage PM2 and the dismantled forms of STIV that we have observed suggest that a completely different mechanism of infection by STIV may be possible (2). The STIV image reconstructions resemble the architecture of PM2, and the lipid cores of STIV and PM2 can both be isolated in vitro (12). Given such similarity between STIV and PM2, we postulate that STIV may proceed through a mechanism of infection similar to that of PM2, in which the capsid shell dismantles from the lipid core to allow for the fusion of the viral and host lipid membranes and the release of the viral genome into the host’s cytoplasm. However, it remains to be explained how the S. solfataricus S layer is rearranged for an injection or fusogenic mechanism to take place.

ACKNOWLEDGMENTS

We thank Sarah Tully for careful reading of the manuscript and providing constructive criticism.

This work was supported by the National Institutes of Health grant R01 GM054076 (to J.E.J. and C.-Y.F.). R.K. was supported by the National Institutes of Health Postdoctoral Fellowship F32 AI065071. A.C.O. and M.J.Y. were supported by National Science Foundation grant MCB 01322156. The 3D reconstruction of STIV was conducted at the National Resource for Automated Molecular Microscopy (NRAMM), which is supported by the National Institutes of Health through the National Center for Research Resources P41 program (RR17573).

REFERENCES


